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May 22, 2015

It has recently come to our attention that the final technical report for contract number N000141210575 held by Prof. Gilbert C. Walker was not submitted. We apologize greatly for this oversight.

Please find enclosed the outstanding report.

Sincerely,

M. Koroniak

Mandy Koroniak
Admin. Asst. to Prof. Walker

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14. ABSTRACT
In this final technical report, we discuss our work as part of the Navy's larger program to reduce operating costs and meet environmental compliance by reducing biofouling to ship hulls through the development and use of improved coating materials. We prepared and characterized phase segregating block copolymer films, which were sent for testing at ONR sites. We laterally resolved the nanoscale surface composition of polymer films from multiple biofouling program contractors.

15. SUBJECT TERMS
antifouling; coatings; block copolymers; IR nanoscale imaging; biocides

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Final Technical Report

Contract Number: N000141210575
Contract Title: Nanostructured Block Copolymer Coatings for Biofouling Inhibition
Grant period: April 1, 2012 – March 31, 2014

Technical Objectives

For optimal performance, ship hulls should be free of biofouling. Our work has been part of a larger program to meet the Navy's needs to reduce operating costs and meet environmental compliance by reducing biofouling to ship hulls through the development and use of improved coating materials. Our objective in the proposed work has been to characterize the function of designed minimally adhesive polymer surfaces based on block copolymers for antifouling applications in a marine environment.

Technical Approach

We prepared phase segregating block copolymer films with specific 10-200 nm length scales of hydrophobicity/hydrophilicity. We made the films using a low VOC process. We characterized these films with cutting edge scanning probe techniques and other techniques as needed. We sent the materials for testing at ONR sites. The testing involved settlement and release of algal spores/sporelings, barnacle cyprids/adults, and tubeworm adults, against these phase segregated block copolymer surfaces. We laterally resolved the nanoscale surface composition of polymer films from multiple biofouling program contractors.

Background

Our previous studies investigated the antifouling properties of a triblock copolymer system PS-b-P2VP-b-PEO in the field in Florida. No settlement of tubeworms or barnacles was recorded on the test surfaces while they occurred on the controls. It was hypothesized that the PS-b-P2Vp-b-PEO material would show antifouling efficacy against algal spore settlement in the laboratory assay. The mechanism would be nanoscale amphiphilicity. Of secondary interest has been the preparation method of the films. The ones used in the study were prepared by an aqueous process, instead of spin casting the film from an organic solvent.

In Birmingham with the Callow group, we have prepared and tested samples of PS (13000)-P2VP(13000)-PEO(36000), with single block PS, P2VP, PEO, and diblock PS-PEO controls, prepared using a phase transfer process to create nanostructured antifouling coatings. Different transfer agents, and concentrations of agents, yielded varying degrees of polymer coverage and nanostructure assembly when applied to a glass slide (characterization was done using an AFM; dry surface, tapping mode). Two of the agents yielded the most consistent and dense coverage and structure: PS-PEO and TWEEN-80 (both 0.1 g/L). Depending on how these coatings perform in the algal settlement tests, various aspects of the process can be altered to increase performance. In summary, the performance was as follows: spore settlement densities on the triblock PS-P2VP-PEO test coatings were lower than on the PS, P2VP, and PEG controls. Spore settlement density was lowest on the diblock PS-PEO coating. There was no difference between spore settlement densities on the PS-P2VP-PEO coatings prepared using two distinct phase transfer agents.

The phase transfer process is illustrated in Figures 1 and 2 below:

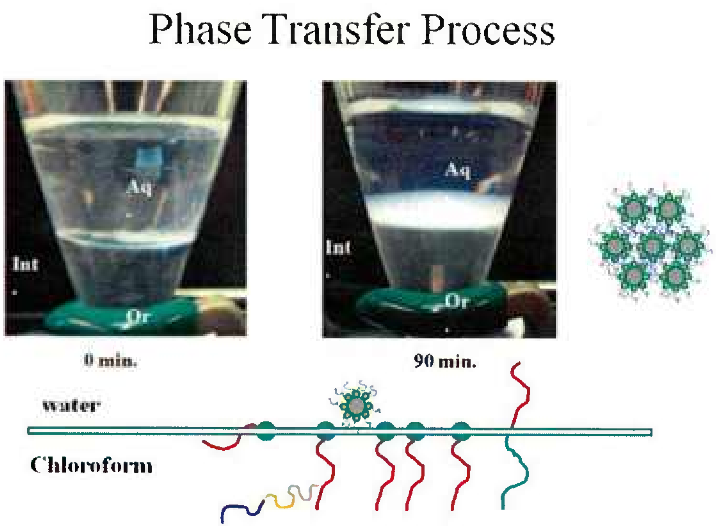


Figure 1 - The prototype phase transfer process uses a small amount of diblock coloymer (0.01% by weight). A micellar nanogel is created.

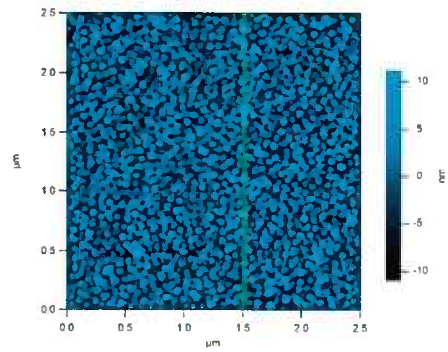


Figure 2 - Films prepared from the nanogel exhibit the nanophase segregation as desired, though the internal morphology is different from that of materials prepared from organic solvents.

We accelerated the phase transfer process from 90 to less than 10 minutes.

In the summaries above, the spore settlement densities on control coatings were in the order PS > P2VP > PEG, as would be anticipated by the contact angle data. Settlement on the PS-PEO control was lower than on the PEG (despite similar contact angles) indicating that the amphiphilic nature and patterning of the coating was deterring spore settlement. The two PS-P2VP-PEO test coatings also had settlement densities that were lower than the PEG control. However, there was little difference between them, suggesting that the different method of phase transfer (PS-PEO vs TWEEN-80) did not affect the surfaces in a way that influenced spore settlement.

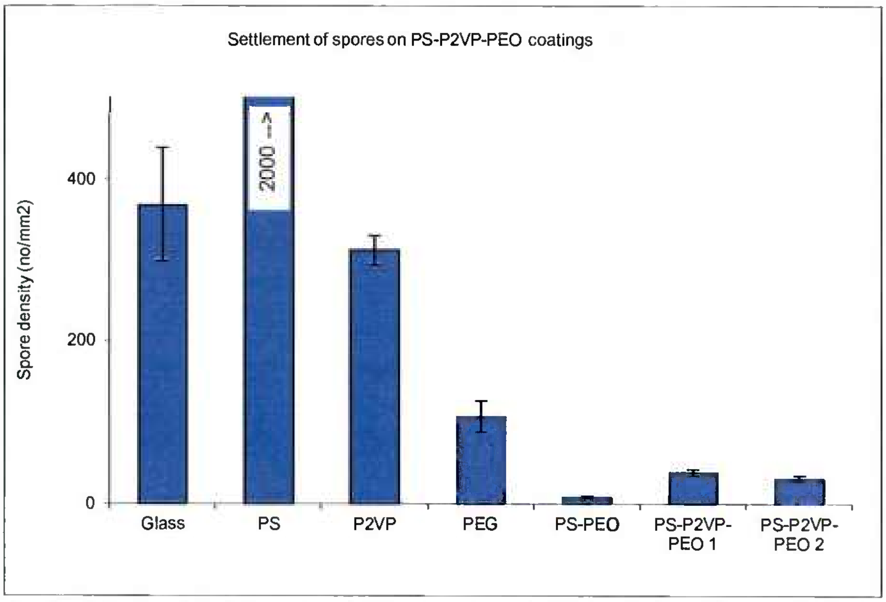


Figure 3 - The density of attached spores PS-P2VP-PEO coatings after 45 minute settlement. Each point is the mean from 90 counts on 3 replicate slides. Bars show 95% confidence limits. Spore settlement density for polystyrene was off the scale at 2000 spores/ mm^2. PS-P2VP-PEO 1 was prepared by PS-PEO phase transfer and PS-P2VP-PEO 2 by TWEEN-80 phase transfer.

The two triblock PS-P2VP-PEO test coatings reduced spore settlement densities compared to the PS, P2VP and PEG controls, however, the lowest settlement density was on the PS-PEO diblock control coating. (See Fig. 3.) It should be pointed out that the settlement density on the PS-PEO and the two PS-P2VP-PEO samples was extremely low (<50 spores/mm^2) and repetition of this measurement was accomplished.

We also developed a tubeworm larvae settlement assay, in collaboration with the Hadfield group. In brief, it involves the use of cesium ions to induce settlement. We have accomplished preliminary studies of the deposited primary glue on simple hydrophilic/hydrophobic surfaces.

Year 1 Progress Statement Summary

Overall, we worked toward the identification of novel, practical materials with enhanced properties that will lead to ship hull coatings with less biofouling, enhancing mission performance, while achieving environmental compliance. In our work on block copolymer coatings fabrication and testing, we have innovated some of better materials to date for algal spore settlement inhibition, with the principle of nanophase segregation leading a way forward. As a matter of broader impact, our aqueous phase transfer process potentially has borne fruit in other technologies, including diagnostics and drug delivery. In our scanned probe microscopy studies on collaborator coatings and marine organisms, we have provided teamwork. We have assisted program members to meet their goals. We have developed and applied for a patent for a new type of IR microscope, which is being commercialized.

We also developed surface-bound biocidal coatings. The scale up to tank-car quantities is straightforward; some of our coatings were applied on 100 meter circumference, 8 meter deep surfaces and show efficacy on nets for 7 months in Bay of Fundy. Performance in Gulf of Mexico was effective for 3 months. Our coatings are being commercialized by Sylleta, a spin-out from the university.

Our 1st year progress was in several areas:

1. Studies of antifouling materials:
    - Fabrication of nanostructured films and testing against marine foulants
        - Florida, Lab assay, and Bay of Fundy
2. Studies of Organisms on model foulants:
    - *H. elegans* studies
3. Testing of other contractor materials
4. Imaging technology.

We applied our organic to aqueous phase transfer process for making block copolymer coatings: Diblock and Triblock copolymers are dissolved in chloroform. An aqueous nanogel is achieved by transferring the copolymer to aqueous phase by using a phase transfer agent (PS-b-PEO) in the aqueous phase (pH1). The process minimizes use of volatile organic compounds (VOC's) and is easy, inexpensive and good for large-scale production. The preliminary conclusions for these materials are as follows:

- Spore settlement densities on the triblock PS-P2VP-PEO test coatings were lower than on the PS, P2VP and PEG controls.
- Spore settlement density was lowest on the diblock PS-PEO coating.
- There was no difference between spore settlement densities on the PS-P2VP-PEO coatings prepared using PS-PEO phase transfer and ones prepared using TWEEN-80 phase transfer.

We also prepared nanopatterned polymers via a thermal imprinting process. We prepared flat PS and P2VP controls and linear nanopatterned samples. The features are summarized in Table 1, with performance illustrated in figure 4.

Sample	Line width (nm)	Groove width (nm)	Depth (nm)	Contact Angle
Polystyrene	-	-	-	88
Polystyrene	200	200	50	94
Polystyrene	700	300	15	94
Polystyrene	900	500	100	103
Poly(2-vinyl pyridine)	-	-	-	41
Poly(2-vinyl pyridine)	200	200	50	38
Poly(2-vinyl pyridine)	700	300	15	39
Poly(2-vinyl pyridine)	900	500	100	38

Table 1

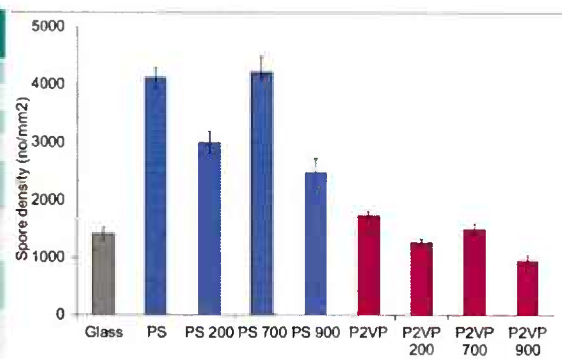


Figure 4

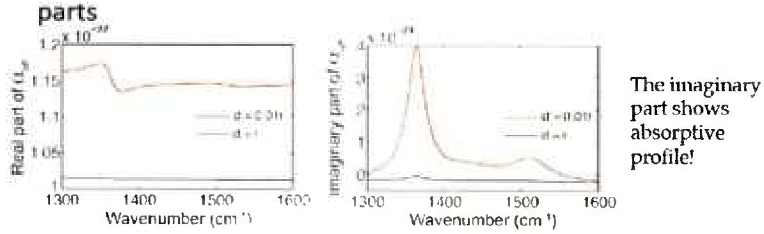
The summary of this work is that block copolymer phase transfer methods can be made to work faster; larger volumes are also not a problem. Performance of these materials looks good in lab – field tests underway. Delamination problems appear resolved. For homopolymers, we find that nanoscale feature variations even on single component materials change settlement.

Imaging:

We made an advance in IR nanoscale imaging. See Figures 5 and 6. Technology licensing by one SPM companies was negotiated.

Our approach, phase controlled homodyne (patent applied for)

– Analyze near-field signal contribution: real and imaginary parts



- Solution: homodyne the imaginary part with a phase controlled reference

$$I_s(\omega) = |\alpha_{eff}E(\omega) + Ae^{i\phi}E(\omega)|^2$$

$$I_s(\omega) = \left((Re\{\alpha_{eff}\} + A\cos\phi)^2 + \boxed{(Im\{\alpha_{eff}\} + A\sin\phi)^2}\right)|E(\omega)|^2$$

Tune the reference phase ϕ to $\pi/2$, imaginary part is amplified

Figure 5 - Proper choice of phase in a reference field of the scattering microscope gives the absorption spectrum

One engineering improvement: phase stabilization feedback device

- Phase drift is the main source of error in the phase controlled homodyne near-field method.
- A phase feedback device has been developed to stabilize the interferometer
- It improves measurement stability
- Applied for a patent for the phase controlled homodyne method

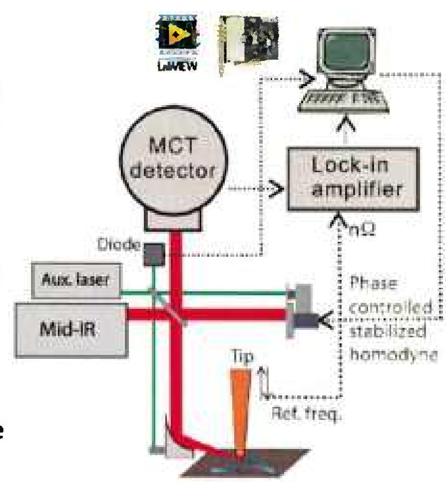


Figure 6 - A feedback loop is a key to making this instrument stable and sensitive. The HeNe light is used to measure the position of the mirror, and keep it fixed so the the phase of the much longer wavelength IR light is controlled in the interferometer.

Spatial resolution estimation: < 15 nm

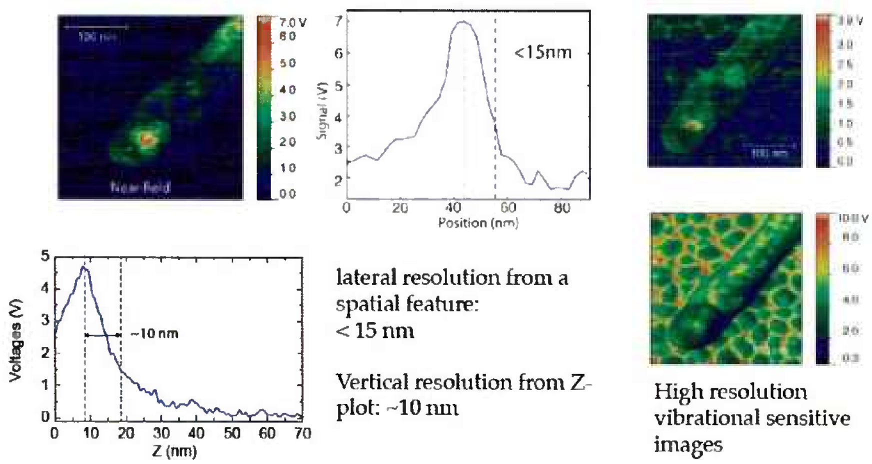


Figure 7 - The instrument provides best-in-world performance. The images are of a boron nitride nanotube. Spatial resolution of less than 15nm is shown.

Nanostructured Foul-release + surface bound biocide antifouling coating technology

We developed a surface bound biocide using a hydrophobic terpenoid in a polymer. Related to this is significant Intellectual Property: 4 Invention disclosures, 2 patents pending, publications and some trade secrets. The pure biocide has been tested by independent labs for toxicity to various mammals and fish, and shown to be safe. Therefore, we have undertaken full scale field trials coating aquaculture cages in Nova Scotia and New Brunswick. These 100 m circumference cages are 10 m deep (shaped like a tuna fish can). Our industrial partner, Cooke Aquaculture, has found the performance, in the half year test authorized by Health Canada, to be better than that for Flexgard. This is because the fouling inhibition was equivalent, and the cleaning of nets after 6 months was much easier than for Flexgard. A company, Sylleta, is trying to commercialize this technology. Cooke made significant cash investments in Sylleta, which continue.

In Figure 8 are the data on the toxicity of the active ingredient (biocide), which is surface tethered in the application. The data are for pure active ingredient. In Figure 9 is a picture of the field application.

Competitive Analysis

Higher numbers indicate more environmentally friendly.
LD50/LC50: concentration at which 50% of test species experience toxic effects.

Ecotoxicity (> the number = safer)	Sylleta Inc. MACROBLOCK I	Flexabar Corp. FLEXGUARD ™
LD50 oral (Rat)	> 5,000 mg/Kg	470 mg/Kg
LC50/96 hrs(Salmonoids)	> 4mg/L	0.06 mg/L
LC50/96 hrs Rainbow Trout	150 mg/L	25.4 mg/L
EC50/48hrs Daphnia	23.63 mg/l	0.011 mg/L
Degradability in Water	Readily Biodegradable	Does not Biodegrade

Figure 8 - Toxicity summary for Macroblock 1 versus Flexguard

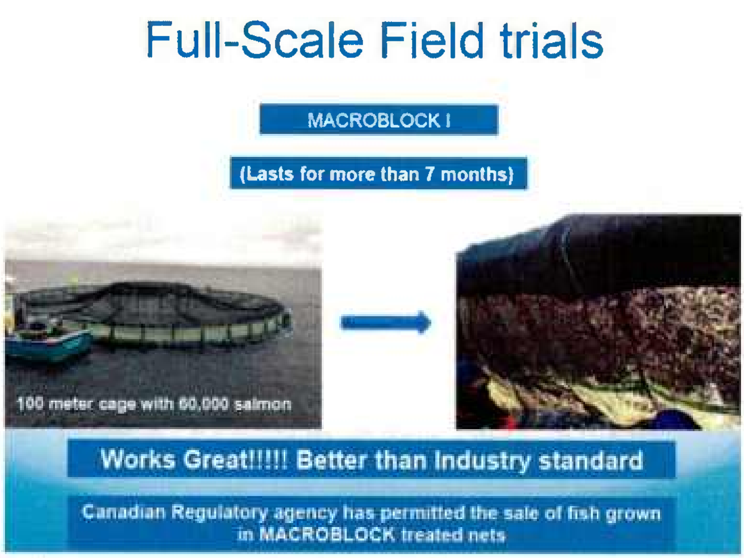


Figure 9 - A picture of a Macroblock treated fish cage in New Brunswick

We made long term tests underway at FIT.

We completed some preliminary tests on clam nets in the Gulf of Mexico, see below. Excellent efficacy was observed after 3 months in that test. See Figure 10.

Figure 10 - (left) Placement of treated net samples in the Gulf of Mexico. (middle) Untreated control. (right) MB2 treated net, showing low fouling ater 3 months immersion.

Year 2 progress:
We explored the role of nanophase size on foulant inhibition. In our scanned probe microscopy studies on collaborator coatings and marine organisms, we provided teamwork. We assisted program members to meet their goals. We have developed and patented a new type of IR microscope, which is being commercialized.

We have also developed and tested several new surface-bound biocidal coatings. The scale up to tank-car quantities of coating is straightforward; some of our coatings have been applied on 100m^2 scale surfaces. We tested these material formulations in the ONR biofouling program test site in Florida.

Our progress lay in several areas:
1. Studies of antifouling materials:
   - Fabrication of nanostructured films and testing against marine foulants
     - Florida, Lab assay, and Bay of Fundy
2. Studies of Organisms on model foulants:
   - *H. elegans* studies
3. Testing of other contractor materials
4. Imaging technology.

Results in previous years showed the ability to inhibit algal spore settlement using diblock copolymers. (see figure 11 below)

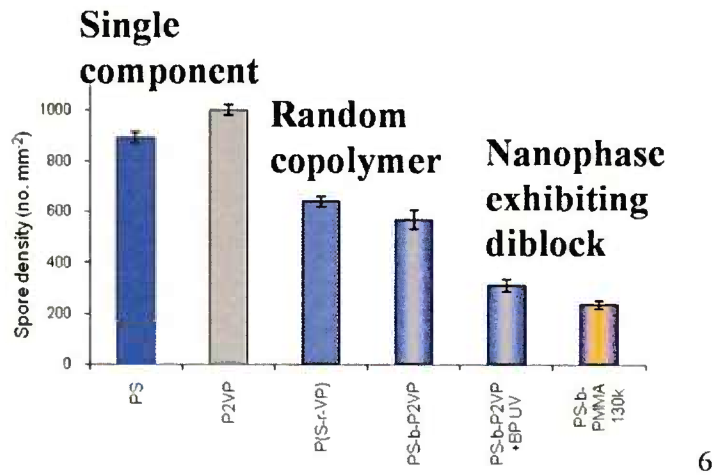


Figure 11

In the second year of the grant year, we tried to find a domain size that would minimize the settlement, but we were unsuccessful (see figure 12 below)

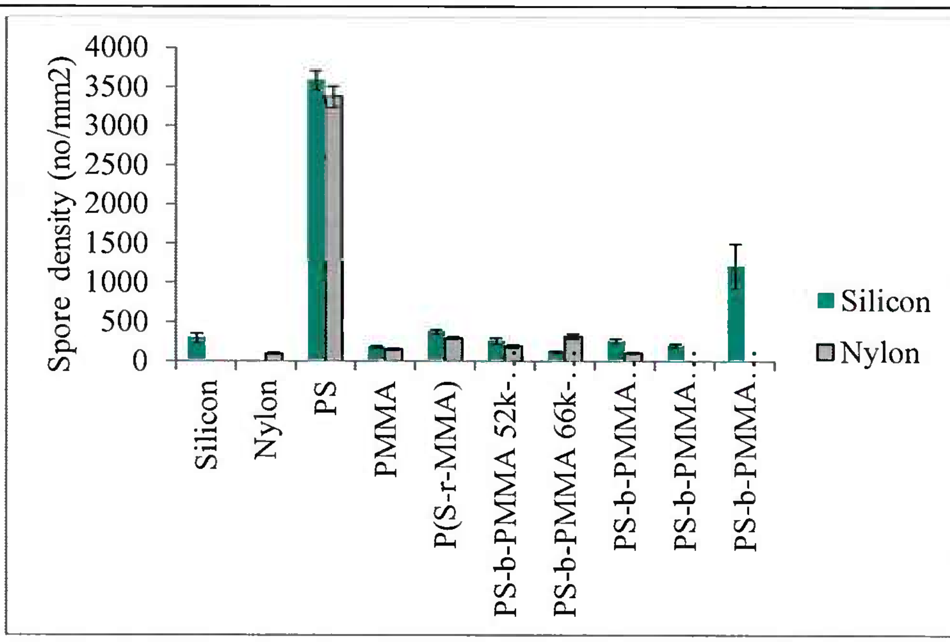


Figure 12 -The settlement density of *Ulva* spores on PS-b-PMMA nanoscale patterned surfaces on silicon and nylon supports. Tuning the separation of nanophase domains of PS-b-PMMA <u>was not</u> as useful as we hoped. Inhibition, but not highly tunable by change of MW ratio

The inhibition of diatoms by the diblocks was not significant (See figure 13).

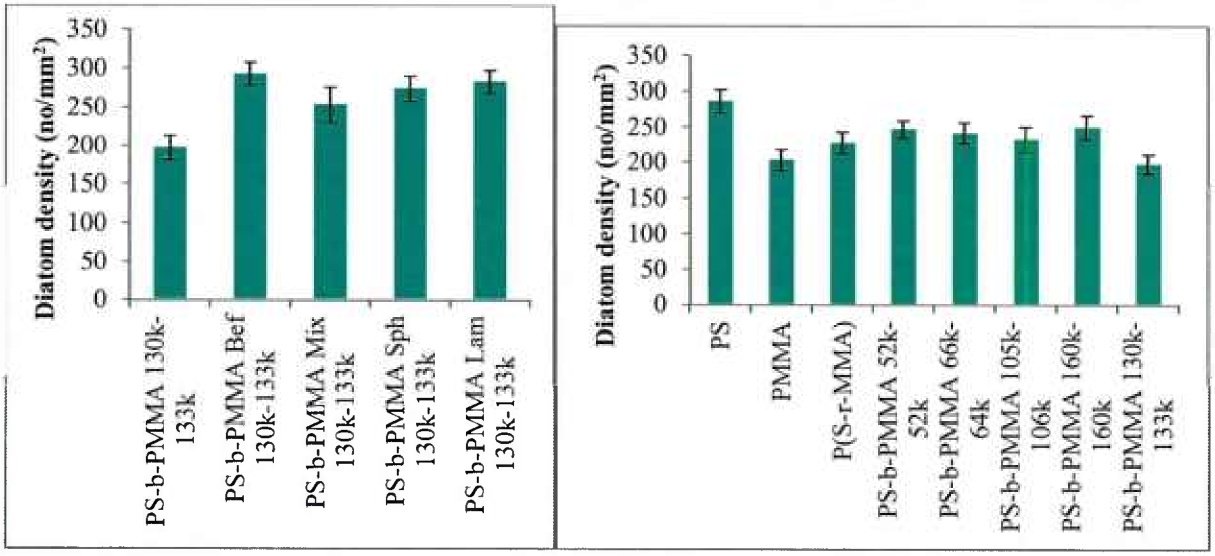


Figure 13 - The initial attachment density of the diatom *Navicula* on PS-b-PMMA coatings after gentle washing on glass-nylon supports. Diatom Settlement: no effect of diblock copolymer

We did have some success with triblocks, and that work is on-going.

We made initial tests of a surface bound biocide, which is less toxic than copper, See Table 2.

Table 2 - Toxicity of biocide, compared with copper standard

Ecotoxicity (> the number = safer)	Sylleta Inc. MACROBLOCKI TM	Flexabar Corp. Flexguard TM
LD50 oral (Rat)	> 5,000 mg/Kg	470 mg/Kg
LC50/96 hrs(Salmonoids)	> 4mg/L	0.06 mg/L
LC50/96 hrs Rainbow Trout	150 mg/L	25.4 mg/L
EC50/48hrs Daphnia	23.63 mg/l	0.011 mg/L
Degradability in Water	Readily Biodegradable (<50 hrs in seawater)	Does not Biodegrade

After 99 days of exposure, we continued to see significant control of biofouling, as compared to copper, see figure 14 below:

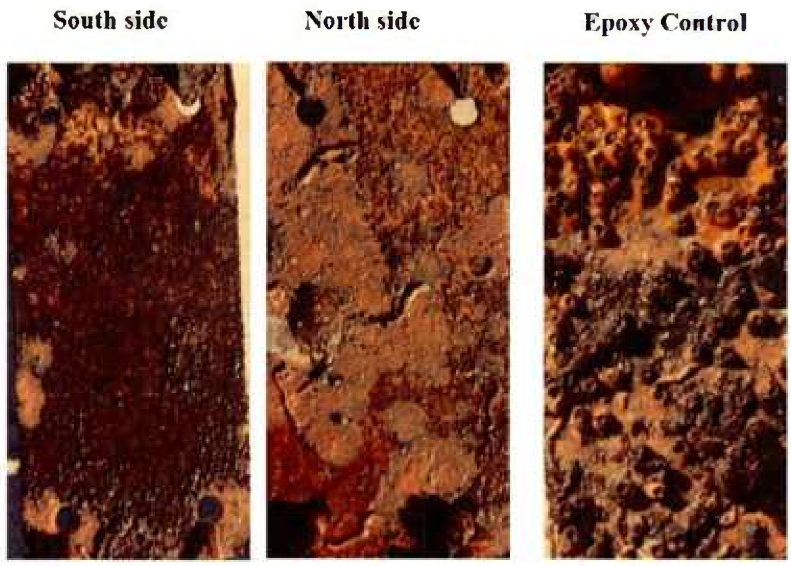


Figure 14 - Inhibition of fouling at the FIT test site, after 99 days exposure.

Quite important, the distribution of species that were present was strongly affected, and barnacles in particular were suppressed (See Figure 15, below)

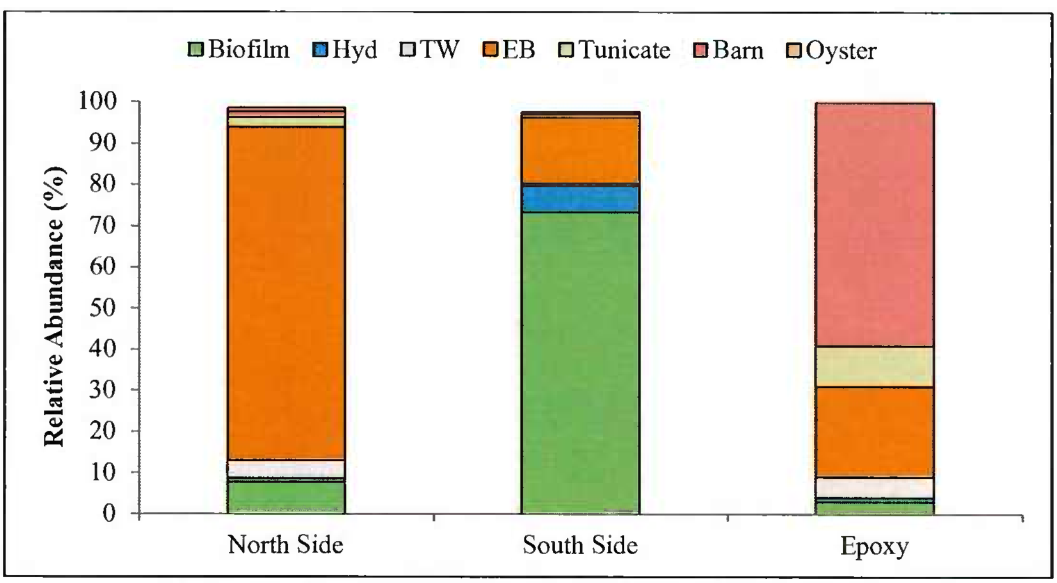


Figure 15 - Barnacles are selectively inhibited by the biocide

Imaging:
We made an advance in IR nanoscale imaging. A patent was granted to us for it. Bruker will sell an instrument based on this, beginning in 2015.

Conclusions and Future work:
While the triblock work is promising, and is worth continuing, a better way forward may be to expand the effort with a surface bound, degradable biocide. We should explore additional ways to control the release, including polymer-based encapsulation IP that we have recently applied for patent protection.